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(54) **T CELL RECEPTORS WITH IMPROVED PAIRING**

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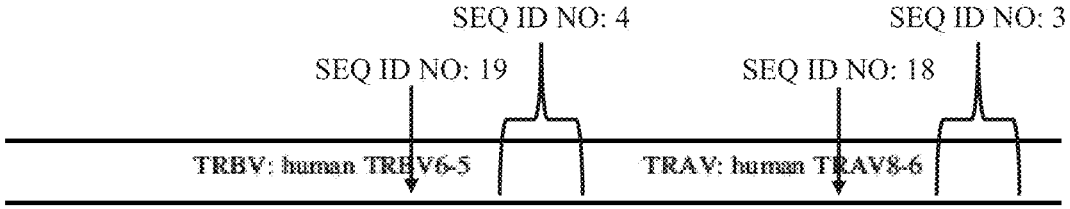
(52) **U.S. Cl.**

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(57) **ABSTRACT**

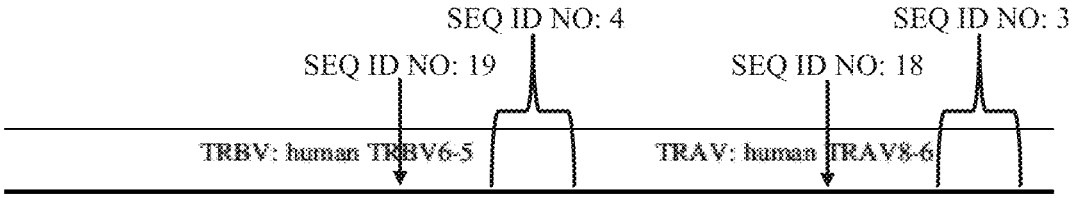
The present invention relates to modified T cell receptor (TCR) α or β chains, or heterodimers comprising the same, wherein in the variable domain of said modified α or β chain, an amino acid at position 44 according to the IMGT numbering is substituted by another suitable amino acid in order to improve pairing of desired chains.

Specification includes a Sequence Listing.



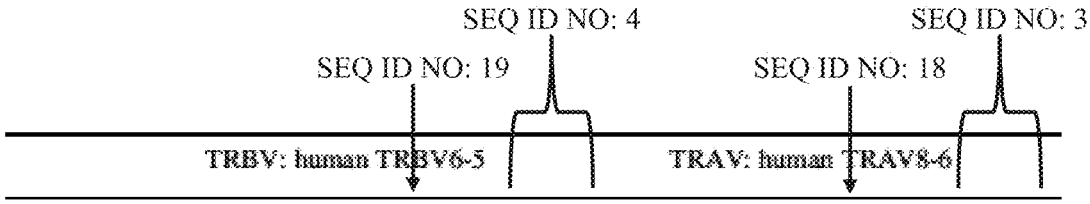
FR1-IMGT	**1**	1	aat	ASN	N	**1**	0	agc	ALA	A
	2	2	gct	ALA	A	**2**	1	cag	GLN	Q
	3	3	ggt	GLY	G	**3**	2	tct	SER	S
	4	4	gtc	VAL	V	**4**	3	gtg	VAL	V
	5	5	act	THR	T	**5**	4	acc	THR	T
	6	6	cag	GLN	Q	**6**	5	cag	GLN	Q
	7	7	acc	THR	T	**7**	6	ctt	LEU	L
	8	8	cca	PRO	P	**8**	7	gac	ASP	D
	9	9	aaa	LYS	K	**9**	8	agc	SER	S
	10	10	ttc	PHE	F	**10**	9	caa	GLN	Q
	11	11	cag	GLN	Q	**11**	10	gtc	VAL	V
	12	12	gtc	VAL	V	**12**	11	cct	PRO	P
	13	13	ctg	LEU	L	**13**	12	gtc	VAL	V
	14	14	aag	LYS	K	**14**	13	ttt	PHE	F
	15	15	aca	THR	T	**15**	14	gaa	GLU	E
	16	16	gga	GLY	G	**16**	15	gaa	GLU	E
	17	17	cag	GLN	Q	**17**	16	gcc	ALA	A
	18	18	agc	SER	S	**18**	17	cct	PRO	P
	19	19	atg	MET	M	**19**	18	gtg	VAL	V
	20	20	aca	THR	T	**20**	19	gag	GLU	E
	21	21	ctg	LEU	L	**21**	20	ctg	LEU	L
	22	22	cag	GLN	Q	**22**	21	agg	ARG	R
	23	23	tgt	CYS	C	**23**	22	tgc	CYS	C
	24	24	gcc	ALA	A	**24**	23	aac	ASN	N
	25	25	cag	GLN	Q	**25**	24	tac	TYR	Y
	26	26	gat	ASP	D	**26**	25	tca	SER	S
CDR1-IMGT	**27**	27	atg	MET	M	**27**	26	tcg	SER	S
	28	28	aac	ASN	N	**28**	27	tct	SER	S
	29	29	cat	HIS	H	**29**	28	gtt	VAL	V
	30	30	gaa	GLU	E	**30**	29	tca	SER	S
	31	*31	tac	TYR	Y	**31**	30	gtg	VAL	V
	32	*	–	–	–	**32**	*31	tat	TYR	Y
	33		–	–	–	**33**	*	–	–	–
	34		–	–	–	**34**	*	–	–	–
	35		–	–	–	**35**		–	–	–
	36		–	–	–	**36**		–	–	–
	37		–	–	–	**37**		–	–	–
	38		–	–	–	**38**		–	–	–

Fig. 1A



FR2-IMGT	39	32	atg	MET	M	39	32	ctc	LEU	L
	40	33	tcc	SER	S	40	33	ttc	PHE	F
	41	34	tgg	TRP	W	41	34	tgg	TRP	W
	42	35	tat	TYR	Y	42	35	tat	TYR	Y
	43	36	cga	ARG	R	43	36	gtg	VAL	V
	44	37	caa	GLN	Q	44	37	caa	GLN	Q
	45	38	gac	ASP	D	45	38	tac	TYR	Y
	46	39	cca	PRO	P	46	39	ccc	PRO	P
	47	40	ggc	GLY	G	47	40	aac	ASN	N
	48	41	atg	MET	M	48	41	caa	GLN	Q
	49	42	ggg	GLY	G	49	42	gga	GLY	G
	50	43	ctg	LEU	L	50	43	ctc	LEU	L
	51	44	agg	ARG	R	51	44	cag	GLN	Q
	52	45	ctg	LEU	L	52	45	ctt	LEU	L
	53	46	att	ILE	I	53	46	ctc	LEU	L
	54	47	cat	HIS	H	54	47	ctg	LEU	L
	55	48	tac	TYR	Y	55	48	aag	LYS	K
CDR2-IMGT	56	49	tca	SER	S	56	*49	tat	TYR	Y
	57	50	gtt	VAL	V	57	*50	tta	LEU	L
	58	51	ggt	GLY	G	58	*51	tca	SER	S
	59	52	gct	ALA	A	59	*52	gga	GLY	G
	60	53	ggt	GLY	G	60		...	...	...
	61	54	atc	ILE	I	61		...	...	...
	62		...	...	...	62		...	...	...
	63		...	...	...	63		...	...	...
	64		...	...	...	64		...	...	...
	65		...	...	...	65		...	...	...
FR3-IMGT	66	55	act	THR	T	66	*53	tcc	SER	S
	67	56	gac	ASP	D	67	*54	acc	THR	T
	68	57	caa	GLN	Q	68	*55	ctg	LEU	L
	69	58	gga	GLY	G	69	*56	gtt	VAL	V
	70	59	gaa	GLU	E	70	*57	gaa	GLU	E
	71	60	gtc	VAL	V	71	*58	agc	SER	S
	72	61	ccc	PRO	P	72	*59	atc	ILE	I
	73	*62	...	...	...	73	*	...	...	...
	74	*63	aat	ASN	N	74	*60	aac	ASN	N
	75	*64	ggc	GLY	G	75	61	ggt	GLY	G
	76	65	tac	TYR	Y	76	62	ttt	PHE	F
	77	66	aat	ASN	N	77	63	gag	GLU	E

Fig. 1B



	78	67	gtc	VAL	V	**78**	64	gct	ALA	A
	79	68	tcc	SER	S	**79**	65	gaa	GLU	E
	80	69	aga	ARG	R	**80**	66	ttt	PHE	F
	81	70	tca	SER	S	**81**	67	aac	ASN	N
	82		---	---	---	**82**	68	aag	LYS	K
	83	71	acc	THR	T	**83**	69	agt	SER	S
	84	72	aca	THR	T	**84**	70	caa	GLN	Q
	85	73	gag	GLU	E	**85**	71	act	THR	T
	86	74	gat	ASP	D	**86**	72	tcc	SER	S
	87	75	ttc	PHE	F	**87**	73	ttc	PHE	F
	88	76	ccg	PRO	P	**88**	74	cac	HIS	H
	89	77	ctc	LEU	L	**89**	75	ttg	LEU	L
	90	78	agg	ARG	R	**90**	76	agg	ARG	R
	91	79	ctg	LEU	L	**91**	77	aaa	LYS	K
	92	80	ctg	LEU	L	**92**	78	ccc	PRO	P
	93	81	tcg	SER	S	**93**	79	tca	SER	S
	94	82	gct	ALA	A	**94**	80	gtc	VAL	V
	95	83	gct	ALA	A	**95**	81	cat	HIS	H
	96	84	ccc	PRO	P	**96**	82	ata	ILE	I
	97	85	tcc	SER	S	**97**	83	agc	SER	S
	98	86	cag	GLN	Q	**98**	84	gac	ASP	D
	99	87	aca	THR	T	**99**	85	acg	THR	T
	100	88	tct	SER	S	**100**	86	gct	ALA	A
	101	89	gtg	VAL	V	**101**	87	gag	GLU	E
	102	90	tac	TYR	Y	**102**	88	tac	TYR	Y
	103	91	ttc	PHE	F	**103**	89	ttc	PHE	F
	104	92	tgt	CYS	C	**104**	90	tgt	CYS	C
CDR3-IMGT	**105**	93	gcc	ALA	A	**105**	91	gct	ALA	A
	106	94	agc	SER	S	**106**	92	gtg	VAL	V
	107	95	agt	SER	S	**107**	93	agt	SER	S
	108	96	tat	TYR	Y					
	109	97	---	---	---					

Fig. 1C

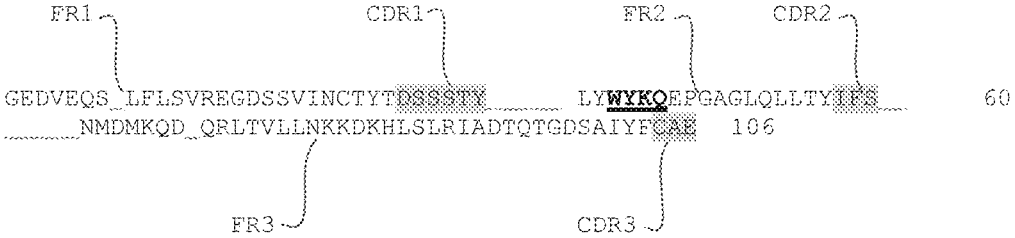

wild type : αQ44-βQ44

Fig. 2A

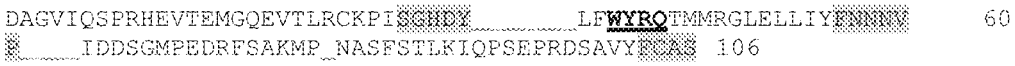
αQ44D-βQ44R
αQ44R-βQ44D
αQ44E-βQ44K
αQ44K-βQ44E
αQ44D-βQ44K
αQ44K-βQ44D
αQ44E-βQ44R
αQ44R-βQ44E
αQ44L-βQ44W
αQ44W-βQ44L
αQ44W-βQ44W
αQ44W-βQ44V

Fig. 2B

SEQ ID NO 1: TCR alpha chain variable domain of R7P1D5 (TRAV5)



SEQ ID NO 2: TCR beta chain variable domain of R7P1D5 (TRBV12-4)



SEQ ID NO 3: TCR alpha chain variable domain of TRAV 8-6



SEQ ID NO 4: TCR beta chain variable domain of TRBV 6-5



Fig. 3

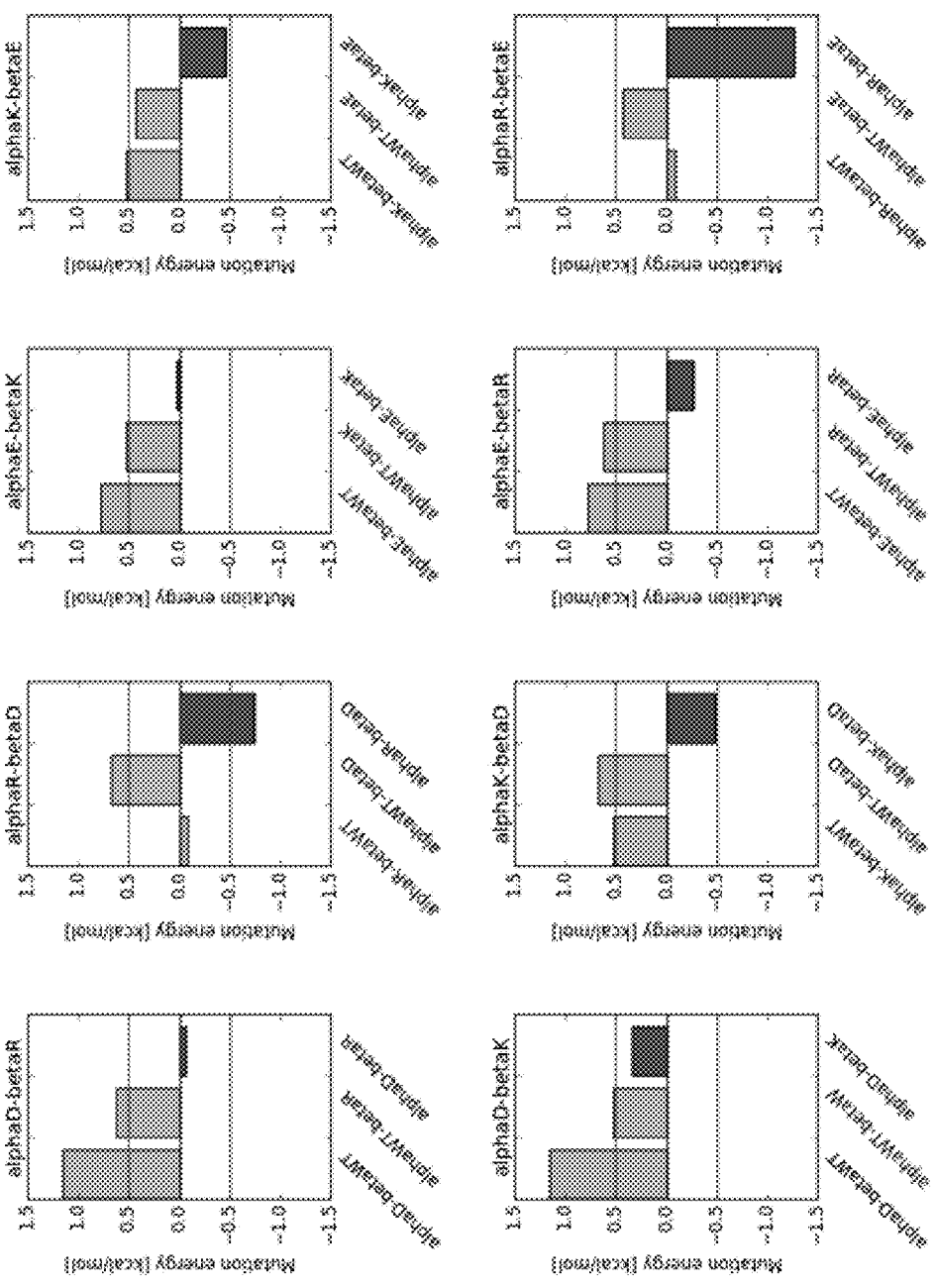


Fig. 4A

Copyright by immatics (MAF-004-07)

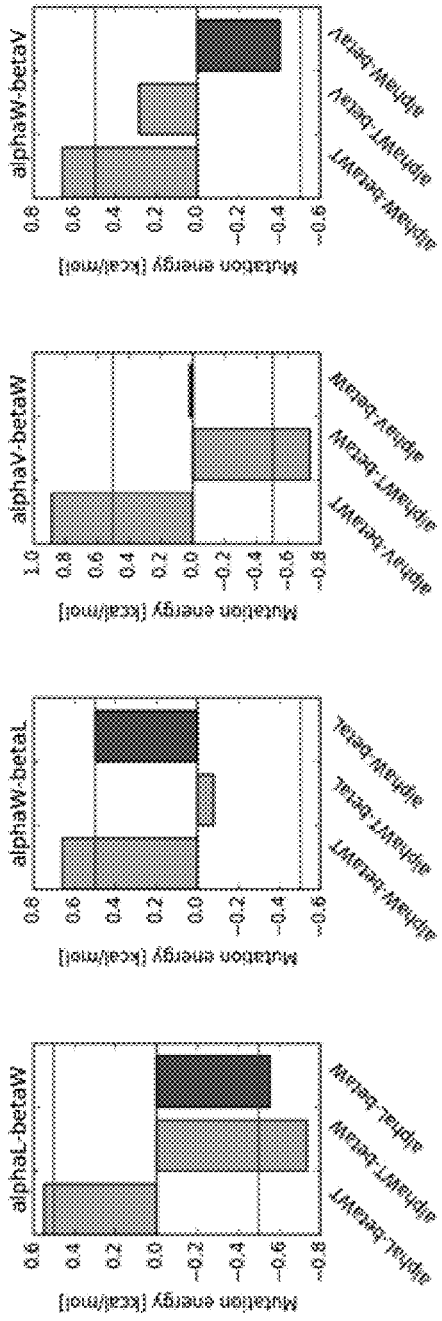

alphaW-betaV
alphaW_betaV
alphaWT_betaV
alphaW_betaWT
Mutation energy [kcal/mol]
alphaV-betaW
alphaV_betaW
alphaWT_betaW
alphaV_betaWT
Mutation energy [kcal/mol]
alphaW-betaL
alphaW_betaL
alphaWT_betaL
alphaW_betaWT
Mutation energy [kcal/mol]
alphaL_betaW
alphaWT_betaW
alphaL_betaWT
Mutation energy [kcal/mol]

Fig. 4B

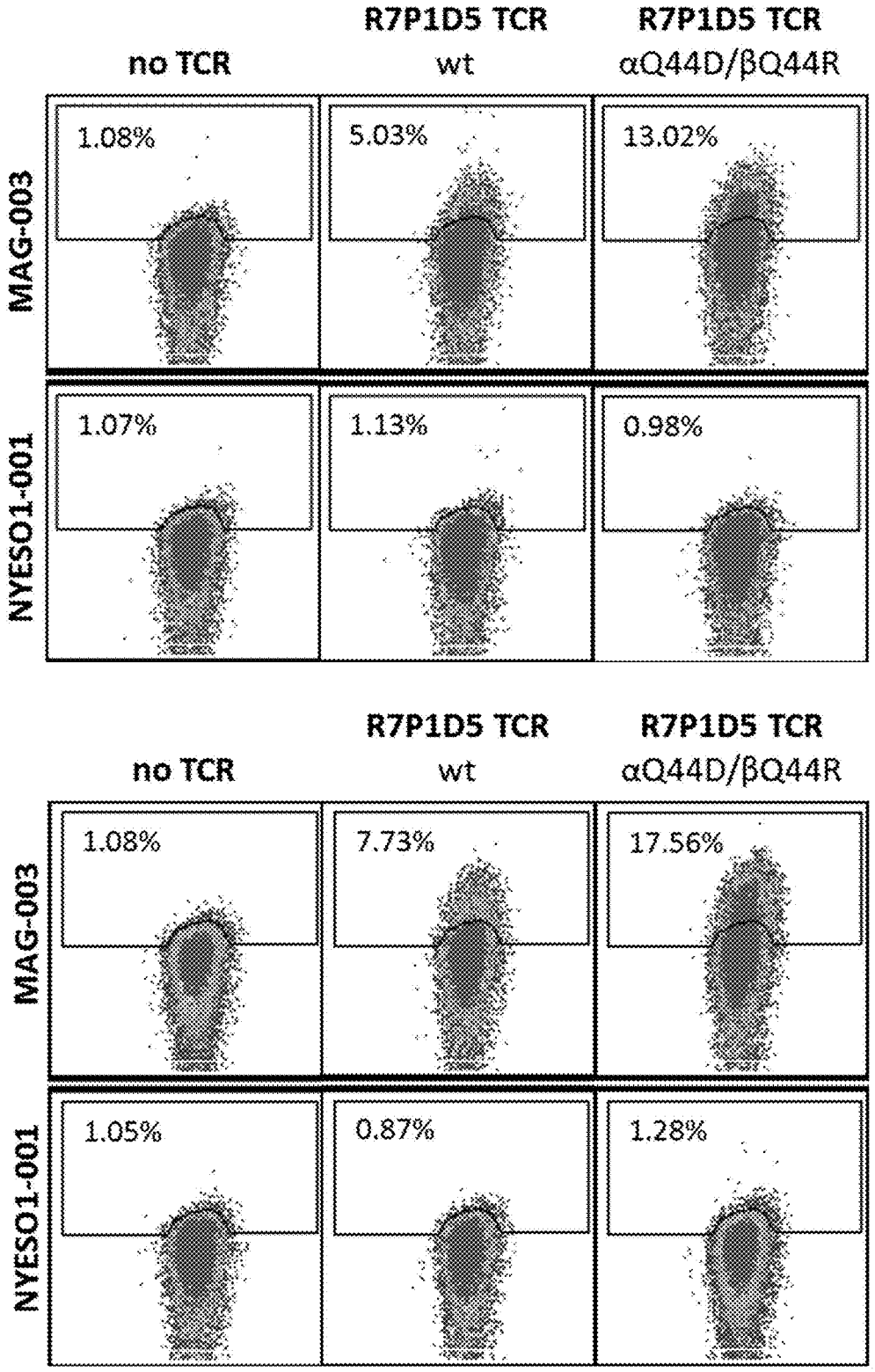

no TCR
R7P1D5 TCR wt
R7P1D5 TCR αQ44D/βQ44R
MAG-003
NYESO1-001
1.08%
5.03%
13.02%
1.07%
1.13%
0.98%
no TCR
R7P1D5 TCR wt
R7P1D5 TCR αQ44D/βQ44R
MAG-003
NYESO1-001
1.08%
7.73%
17.56%
1.05%
0.87%
1.28%

Fig. 5

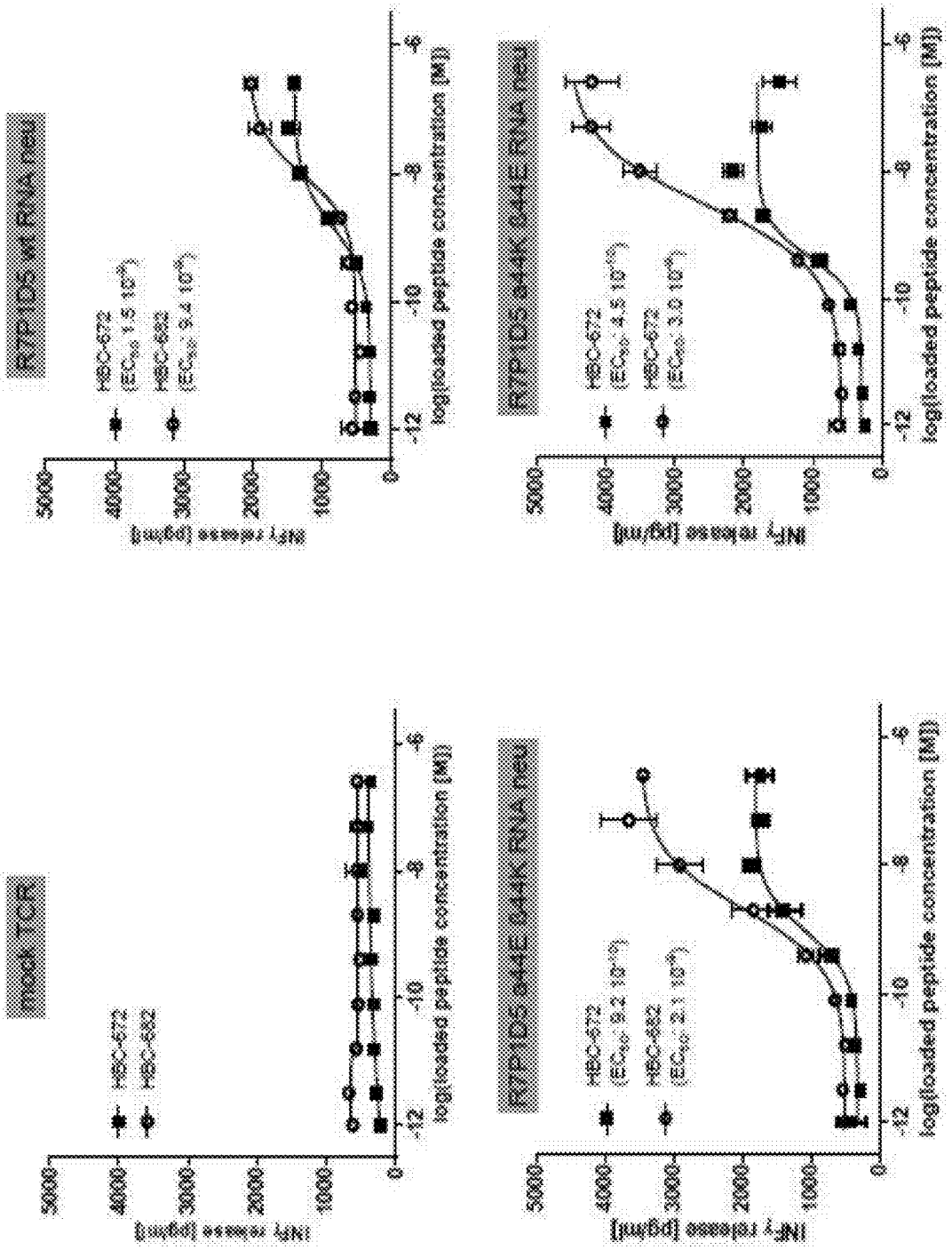

mock TCR
R7P1D5 wt RNA (nM)
R7P1D5 B44K B44E RNA (nM)
R7P1D5 B44E B44K RNA (nM)
HSC-672
HSC-692
IFNγ release [pg/ml]
logl/loaded peptide concentration [M])

Fig.6

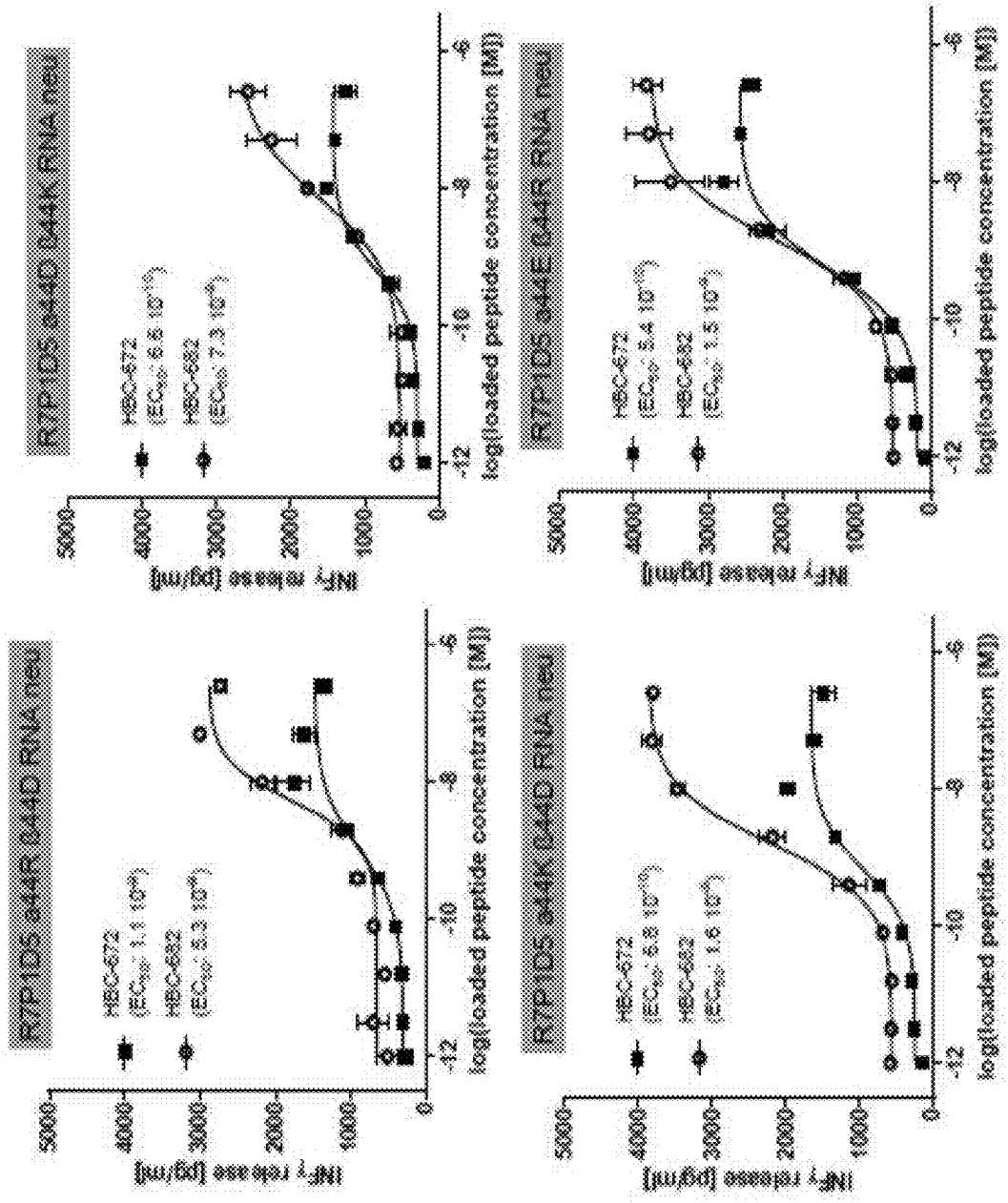

R7P1DS a44R (44D RNA neo)
R7P1DS a44D (44K RNA neo)
R7P1DS a44K (44D RNA neo)
R7P1DS a44E (44R RNA neo)
HEC-672
HEC-682
IFNγ release [pg/ml]
log(loaded peptide concentration [M])

Fig. 6, cont'd

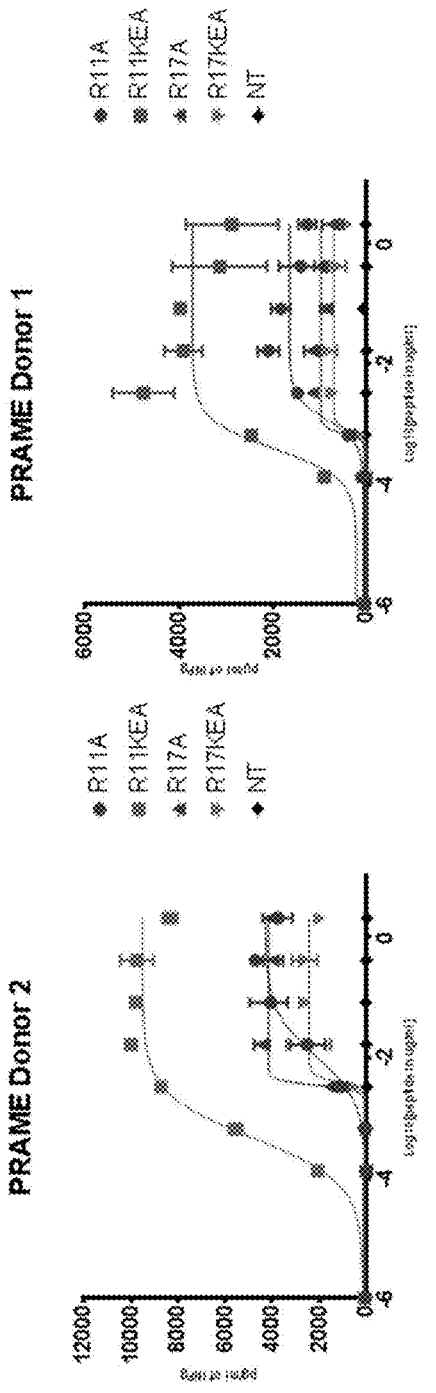

PRAME Donor 1
PRAME Donor 2
R11A
R11KEA
R17A
R17KEA
NT
6000
4000
2000
12000
10000
8000
0
-2
-4
-6

Fig.7

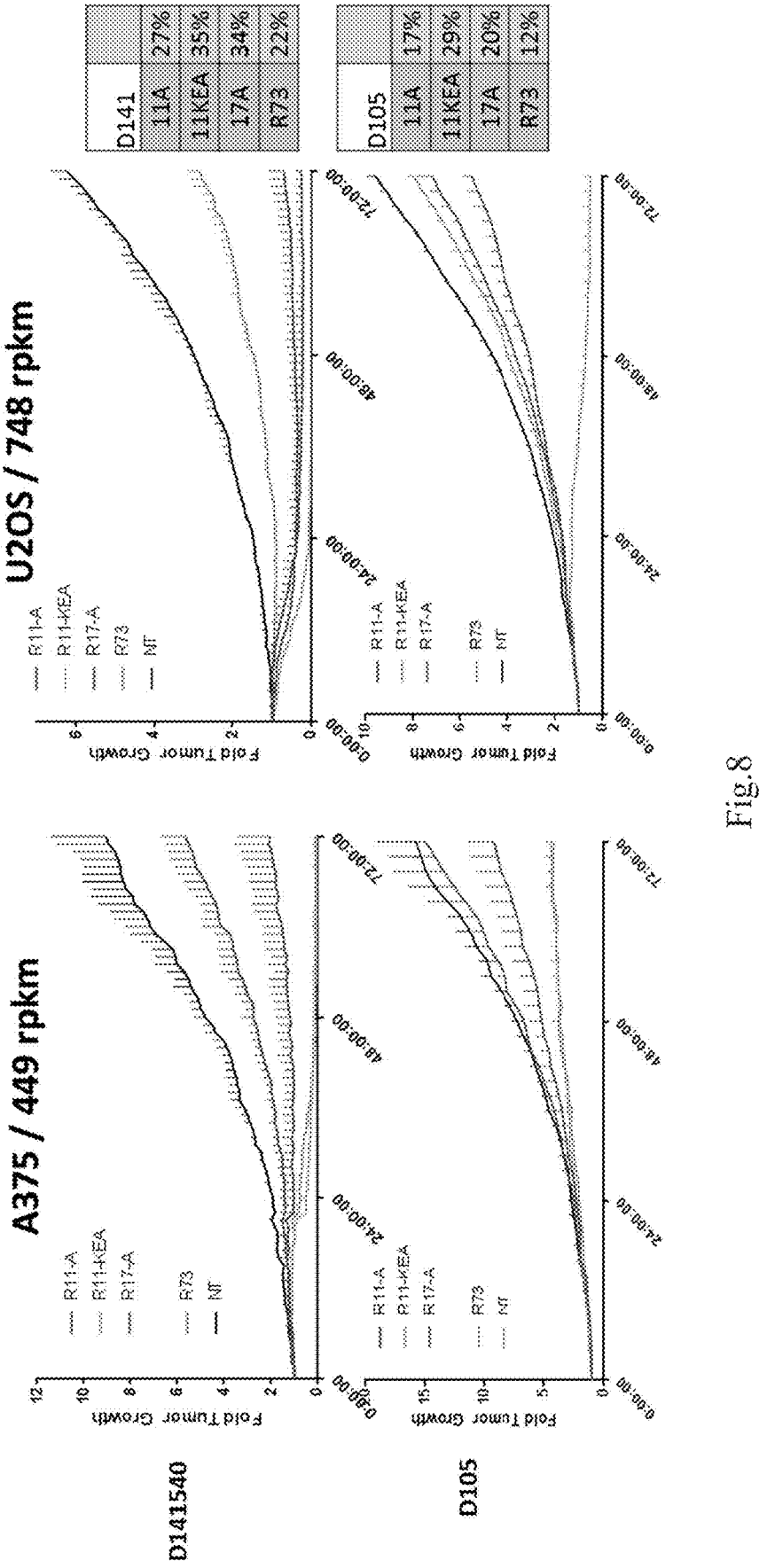

D141540
D105
A375 / 449 rpkm
U2OS / 748 rpkm
R11-A
R11-KEA
R17-A
R73
NT
Fold Tumor Growth
0:00:00
24:00:00
48:00:00
72:00:00
D141
11A 27%
11KEA 35%
17A 34%
R73 22%
D105
11A 17%
11KEA 29%
17A 20%
R73 12%

Fig.8

T CELL RECEPTORS WITH IMPROVED PAIRING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of U.S. patent application Ser. No. 17/354,555, filed on 22 Jun. 2021, which is a continuation application of U.S. patent application Ser. No. 15/833,778, filed 6 Dec. 2017, now U.S. Pat. No. 11,072,645, issued on 27 Jul. 2021, which claims priority to U.S. Provisional Application No. 62/497,895, filed 8 Dec. 2016 and German Patent Application No. 10 2016 123 893.7, filed 8 Dec. 2016, the entire contents of each of which are incorporated herein by reference.

[0002] This application is also related to PCT/EP2017/081745 filed 6 Dec. 2017, the content of which is incorporated herein by reference in its entirety.

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REFERENCE TO SEQUENCE LISTING SUBMITTED AS AN XML FILE (.XML)

[0004] A Sequence Listing is submitted herewith as an XML file named "3000058-007003_Sub_Sequence_Listing_ST26" created on 27 Jan. 2025 and having a size of 23,632 bytes as permitted under 37 C.F.R. § 1.821 (c). The material in the aforementioned file is hereby incorporated by reference in its entirety.

BACKGROUND

Field of the Invention

[0005] The present invention relates to modified T cell receptor (TCR) α or β chains, or heterodimers comprising the same, wherein in the variable domain of said modified α or β chain, an amino acid at position 44 according to the IMGT numbering is substituted with another suitable amino acid, in order to improve pairing of desired chains.

Description of Related Art

[0006] The adaptive immune system consists of antibodies, B cells, and CD4' and CD8' T cells. These enable a highly specific response against a particular immunogenic target. T cell receptors (TCRs) are disulfide-linked membrane-anchored heterodimeric proteins that normally consist of the highly variable alpha (α) and beta (β) chains expressed as part of a complex with the invariant CD3 chain molecules on the surface of T cells. An important step in the process of forming the TCR heterodimer is called "pairing". T cells expressing paired receptors are referred to as α:β (or a3) T cells, although a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred to as γδ T cells.

[0007] TCRs are located on the surface of T cells, and as antigen receptor molecules are responsible for the recognition of suitably processed antigen presented to T cells by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APCs), leading potentially to the activation of the T cell and an immune response to the antigen.

[0008] Each chain of the TCR is composed of two extracellular domains: A variable (V) region and a constant (C) region, both belonging to the immunoglobulin superfamily (IgSF) domain forming antiparallel β-sheets. The constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the variable region binds to the peptide/MHC complex of an antigen presenting cell.

[0009] The variable domains of both the TCR α-chain and β-chain each have three hypervariable or complementarity determining regions (CDRs), which form the antigen binding site. The variable region of the β-chain has an additional area of so-called hypervariability (HV4) that does not normally contact antigen and, therefore, is not considered a CDR. Importantly, CDRs 1 and 2 of both chains are germline encoded while CDR3 α and β are largely non-template encoded but produced by somatic recombination (Davis & Bjorkman 1988) In humans, the diversity of TCR molecules is achieved by the αβ pairing of a set composed 47 Vα and 54 Vβ sequences that are combined to achieve the final diversity of CDR3 lengths and sequences.

[0010] CDR3 are the main CDRs responsible for recognizing said processed antigen, although CDR1 of the α-chain has also been shown to interact with the N-terminal part of the antigenic peptide (Cole et al. 2009), whereas CDR1 of the β-chain only interacts occasionally with the C-terminal part of the peptide.

[0011] CDR2 is thought to mostly recognize the MHC. The HV4 region of the 3-chain is not thought to participate in antigen recognition, but has been shown to interact with so-called superantigens (Li et al. 1998). Despite the commonly accepted paradigm of CDR1 and CDR2 binding mostly to the MHC and CDR3 to the antigenic peptide, some studies have revealed the true complexity of antigen recognition by the TCR, by showing that all CDRs regions can occasionally interact both with the antigen and the MHC (Burrows et al. 2010, Roomp et al. 2011).

[0012] The adoptive transfer approach was developed to use a mechanism for cancer therapy (Rosenberg et al. 1988), whereby T cells transduced with the genes encoding for the α and β chains of a tumor-specific T cell receptor (TCR) mediate anti-tumor immunity in patients. In recent years, this approach has been the center of a lot of attention. As one strategy, TCR gene therapy provides patients with autologous T cells that are thus genetically engineered with transgenic TCR chains. This technique provides a promising approach for the treatment of cancer and tumors. For doing so, TCR alpha and beta chains detecting/binding a specific antigen-MHC complex are cloned into a wildtype T cell taken from a patient. The transgenic T cell is then proliferated in vitro, and the proliferated cells are given back to the patient, in order to provide an immune response against e.g. the tumor.

[0013] In effect, the transgene T cells thus produced both expresses a wildtype TCR having wildtype alpha and beta chains, and the transgenic TCR with the alpha and beta chains specific for the respective recombinant antigen-MHC complex. Both the wildtype alpha and beta chains and the transgenic alpha and beta chains are usually still capable of cross-pairing with each other (Shao et al. 2010). This

undesired possibility is called "TCR mispairing", and is a recognized problem in the field of TCR (gene) therapy.

[0014] The mispairing and the incorrect pairing between a transgenic TCR α or β chain and an endogenous TCR β or α chain, respectively, results in a reduced surface expression of the transgenic TCRαβ heterodimer, which in turn reduces the functional avidity of the modified T cells. Furthermore, T cells expressing mispaired TCRs and expanded under high IL-2 conditions were demonstrated to induce graft-versus-host disease (GvHD) in a preclinical model (Bendle et al, 2009).

[0015] Some strategies for the optimization of transgenic TCR α and β pairing in order to enhance the functional avidity of therapeutic T cells have been discussed, e.g., in Govers et al. (2010).

[0016] Among these possibilities to avoid mispairing are the following:

1. Murinized TCRs: In this approach, human TCRα and β constant chains are replaced by the corresponding murine domains. Although human and murine TCR-C domains show a high degree of homology, small differences affect the stability of TCR/CD3 interactions and hence TCR surface expression levels.
2. Cysteine-modified TCRs: This approach introduces cysteine amino acids at a structurally favorable position, and hence allows the formation of an additional disulfide bridge and promotes correct pairing between the two TCR chains. Site-directed mutations of e.g. T48C in the TCR alpha constant chain and S57C in the TCR beta constant chain resulted in a TCR heterodimer linked by two interchain bonds (i.e., an introduced disulfide bridge plus an endogenous transmembrane disulfide bridge (position No. 95 in the alpha constant domain and position No. 131 in the beta constant domain).
3. Domain swapping: Constant domains are swapped between the α and β chains of a tumor-specific T cell receptor, creating a domain-swapped (ds)TCR. When correctly paired, these dsTCR chains retain all domains necessary to recruit CD3 proteins, to express on the T cell surface, and to mediate functional T cell responses upon engaging a target antigen. By contrast, mispaired TCRs containing one dsTCR chain and one wild-type TCR chain lack key domains necessary for CD3 recruitment, export, and signaling, and thus are unable to mediate deleterious autoimmunity.
4. Exclusive TCR heterodimers: In this approach, sterical and electrostatic forces are exploited to facilitate correct pairing between TCR alpha and beta transgenes and at the same time inhibit pairing between exogenous and endogenous TCR alpha and beta chains. One example uses site-directed mutations to introduce an S85R into the alpha constant domain and R88G in the beta constant domain, in order to obtain the required changes in electrostatic charges, and hence generate a reciprocal 'knob-into-hole' configuration, which allegedly minimally distorts secondary and tertiary structures.
5. The use of chimericTCR-CD3ζ chain having each TCR chain fused to a CD3ζ molecule.
6. The use of single-chain TCRs wherein the Vα of a defined TCR is fused to the beta chain using a flexible peptide linker.
7. The use of shRNA sequences or zinc finger nucleases to knock down the expression of the endogenous TCR.

[0017] Another approach was proposed by O'Shea et al. (1993) who designed a pair of peptides, termed "velcro", that were able to pair with one another due to favorable electrostatic interactions in the heterodimeric state. The authors demonstrated that the two peptides are predominantly unfolded in isolated form but associate preferentially to form a stable parallel, coiled coil heterodimer when mixed. This approach was also applied by Chang et al. (1994) in order to produce soluble TCR in which heterodimeric complex was favored by fusing the peptides to truncated alpha and beta chains respectively.

[0018] WO 2014/153470 A2 discloses methods and compositions for modifying TCR genes, using nucleases (zinc finger nucleases or TAL nucleases) to modify TCR genes by targeted disruption.

[0019] WO 2014/083173 relates to a method for the production of novel T cell receptors which provide a reduced risk of adverse events in immune therapy, specifically in adoptive T cell transfer.

[0020] WO 2016/071343 A1 relates to modified T cell receptors (TCRs) and to their use in adoptive cell therapy (ACT), in particular for the transfer of T lymphocytes. The TCRs are mutated in the transmembrane regions of the alpha and beta chains with mutations favoring the correct TCR chain pairing.

[0021] Although promising, several hurdles, including the proper expression of the exogenous TCR, have hampered the clinical impact of the above approaches. Insufficient clinical responses indicate that many problems are still to be solved. As T cell functional avidity is dictated mainly by both TCR affinity and the number of TCR molecules expressed, much efforts have been devoted to improving these biophysical properties in TCR-engineered cells using two important approaches: (a) the improvement of TCR affinity and (b) the enhancement of TCR expression. To improve TCR affinity, attempts have been made to select high affinity receptors or to enhance the affinity of the transferred receptor by point mutations. Alternatively, various approaches have been devised to increase the number of TCRs on the surface of transduced cells. These include engineering expression vectors, using of co don-optimized TCR sequences, eliminating glycosylation sites and improving pairing of the introduced TCR chains.

SUMMARY

[0022] There is therefore still the need to provide approaches in order to effectively avoid TCR mispairing. These further approaches should be easy to introduce, and efficiently reduce the occurrence of mispaired TCR heterodimers, while increasing the abundance of correctly paired TCR heterodimers, i.e., pairs of transgenic alpha and beta chains in respectively modified T cells. Also, a methodology requiring minimal manipulation of TCR chains and host T cells would be desirable.

[0023] These and further objects are solved by methods and means according to the present invention.

[0024] According to the first aspect of the present invention, a modified T cell receptor (TCR) α or β chain, or a fragment or derivative thereof is provided that maintains the ability to bind to an antigen-MHC complex, wherein, in the variable domain of said modified α or β chain, a Q or any other amino acid at position 44 according to the IMGT numbering is substituted with another suitable amino acid. Suitable amino acids, as is shown also in the following, maintain the pairing specificity of the TCR α and β chains

as modified, while reducing the pairing with an undesired α or β chain, e.g. a non-modified chain.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0026] FIGS. **1**A, **11**B, **1**C, **2**A, **2**B, and **3**-**8** depict embodiments of the disclosure as disclosed herein.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

[0027] Because it is located in the FR2 region, position 44 is known to not be directly in contact with the target of the TCR binding site. It is therefore assumed that a substitution at position 44 would not be crucial for and/or interfere with a specific target epitope recognition.

[0028] Amino acids according to the invention can be selected from the 20 α-amino acids as used in living organisms (L-amino acids). "Suitable" amino acids shall include both these amino acids (i.e. the 20 α-amino acids as used in living organisms, and L-amino acids), as well as "unusual" amino acids (e.g. β-amino acids, or D-amino acids) or modified amino acids. Preferred are the 20 α-amino acids as used in living organisms.

[0029] The numbering of the amino acid residues in the TCR α chain and β chain is according to the IMGT standard, as disclosed in Lefranc et al. (2001). The IMGT standard is a universal system of rules to assign unambiguous numbers to amino acid residues in immunoglobulin molecules, including TCR α and β variable chains. See FIGS. **1** A to C for a comparison of the IMGT numbering (bold) to the Kabat numbering. In FIG. **1** B, Q44 in the α chain (TRAV) and β chain (TRBV) are marked in gray. The numbering according to the IMGT standard may deviate from the naïve numbering of a given amino acid sequence of a TCR variable domain, in particular due to blanks in the CDR sequences, as can be seen in FIG. **1** A-C.

[0030] Q44 according to the IMGT numbering is a highly conserved residue in the Framework 2 (FR2) region, and is shared by almost all TCR α chains, except of TRAV2, TRAV24, TRAV29/DV5 and TRAV40, and is shared by all TCR β genes, except of TRBV14 (Lefranc et al, 2001, Strong et al., 1999). Furthermore, Q44 is very often part of a 4 AA motif comprising WYXQ, with X being, for example, R, V, K or Q (Lefranc et al, 2001).

[0031] Knies et al. (2016) describe that an optimized single-chain TCR (scTCR) inhibits residual TCR mispairing in order to accomplish safe adoptive immunotherapy for bulk endogenous TCRα/β-positive T-cells. Prevention of residual mispairing was achieved by a novel artificial disulfide bond designed between the Vα-domain and the 3'-tail of the linker close to Vβ in a 3-domain scTCR.

[0032] Hoffmann et al. (2015) describe a systematic bioinformatics analysis of the structural characteristics of bound and unbound TCR molecules focusing on the Vα/Vβ relative angle and flexibility. Their results demonstrated the importance of this angle for signaling, as several distinct Vα/Vβ-angle based structural clusters could be observed and larger angle flexibilities exist for unbound TCRs than for bound TCRs. A unique center of rotation was identified and the core region around this point is situated at the center between the Vα and Vβ domains, very close to the positions 44 of both Vα and Vβ.

[0033] Substitutions in the context of the present invention can be generated with standard methods of protein mutagenesis, like, e.g., site-directed mutagenesis or random mutagenesis of the encoding DNA or cDNA, or genome editing technologies, like CRISPR Cas, TALEN, ZFN, Argonaute (NgAgo) or CRISPR Cpf1. Further, such substitution can be carried out by simply synthesizing the encoding gene, cDNA or mRNA. Nowadays, service providers offer the synthesis of a nucleic acid of a given sequence.

[0034] Methods of random mutagenesis or site directed mutagenesis leading to site-specific amino acid substitutions are well known to the skilled person, and are for example disclosed in Labrou et al. (2010) and Trehan et al. (2016).

[0035] Methods of genome editing to modify a given amino acid sequence are well known to the skilled person (e.g., CRISPR Cas, TALEN, ZFN, Argonaute (NgAgo) or CRISPR Cpf1), and are for example disclosed in Maeder & Gersbach (2016). Methods of synthesizing genes are well known to the skilled person, and are for example disclosed in Hughes et al. (2011). The disclosure of these references shall be deemed as incorporated herein by reference in their entireties.

[0036] As discussed above, some prior art methods suggest the modification of the TCR α and/or β chain by introducing murine constant chains. This approach has the risk of increased immunogenicity due to non-human sequences, which is avoided by the methods and products of the present invention.

[0037] Furthermore, the inventors show that the position 44 substitution can have a moderate impact on the binding to the target peptide (i.e., the affinity to the target antigen/MHC complex), which seems due to the fact that, in the tertiary structure of the α and β variable domains, Q44 is maximally distal from the region that is formed by the CDRs.

[0038] According to one embodiment, the modified α chain and β chain do not have any substitutions in their constant domains, and/or in their transmembrane domains, as compared to the respective unmodified receptors as identified and/or isolated. Because the constant domain contains a large and highly conserved interface between TCR α and β chain, the impact of even slight modifications would be difficult to circumvent, in terms of pairing behavior, stability and immunogenicity, compared to the replacement of positions in the variable domain.

[0039] According to one embodiment, an α chain with position 44 substitution preferably pairs with a T cell receptor (TCR) β chain of which position 44 in the variable domain is substituted by a suitable amino acid. Likewise, in another embodiment, a β chain with position 44 substitution preferably pairs with a T cell receptor (TCR) α chain in the variable domain of which position 44 is substituted by a suitable amino acid.

[0040] Hence, in a T-cell that has been modified to express a second, heterologous or transgenic TCR bearing the claimed substitutions, mispairing between a transgenic α chain and an endogenous β chain, or vice versa, is reduced or even avoided.

[0041] In such way, an increased abundance of correctly paired transgenic TCRα/β heterodimers can be achieved, which improves the overall avidity of the modified T-cells

and avoids adverse reactions, like the induction of graft-versus-host disease (Bendle et al, 2009).

[0042] According to one embodiment of said recombinant T cell receptor (TCR) heterodimer, in at least the α or β chain, the amino acid as present at position 44 in the variable domain is substituted by one amino acid selected from the group consisting of Q, R, D, E, K, L, W, and V.

[0043] According to one further embodiment, the α chain has a variable domain sequence comprising at least the framework regions of a sequence sharing (having) at least 95% sequence identity with SEQ ID NO 1 or 3, wherein Q, or any other amino acid occurring at position 44 in the variable domain thereof is substituted by another suitable amino acid as disclosed herein, or the β chain has a variable domain sequence comprising the framework regions of a sequence sharing (having) at least 95% sequence identity with SEQ ID NO 2 or 4, wherein Q, or any other amino acid occurring at position 44 in the variable domain is substituted by another suitable amino acid as disclosed herein.

[0044] Preferably, said α or β chains have a variable domain sequence comprising at least the framework regions of a sequence sharing (having) at least 96%, more preferably 97%, even more preferably at least 98%, even more preferably at least 99% or, most preferably 100% sequence identity with SEQ ID NO: 1 or 3, or SEQ ID NO: 2 or 4, respectively.

[0045] SEQ ID NO: 1 shows the amino acid sequence of the TCR α chain variable domain of TCR R7P1 D5 (also known as TRAV5, Lefranc et al. 2001). SEQ ID NO: 2 shows the amino acid sequence of the TCR β chain variable domain of R7P1 D5 (also known as TRBV12-4, Lefranc et al. 2001). R7P1 D5 is disclosed in U.S. provisional application 62/308,944, the content of which is herein incorporated by reference. R7P1 D5 binds to the peptide called "MAG-003" when bound to the MHC. e.g., of an antigen presenting cell. MAG-003 comprises the amino acid sequence according to the following general formula I (SEQ ID NO: 17):

$$X_1X_2LEHVVRX_3$$

wherein X_1 is selected from the amino acids K and Y, X_2 is selected from the amino acids V, L and A, and X_3 is selected from V, L, A, and I.

[0046] SEQ ID NO: 3 shows the amino acid sequence of the TCR α chain variable domain TRAV 8-6 (Lefranc et al. 2001). SEQ ID NO: 4 shows the amino acid sequence of the TCR β chain variable domain TRBV 6-5 (Lefranc et al. 2001).

[0047] Note that in FIGS. **1** and **3**, the sequences are shown with the IMGT numbering, which deviates from the naïve numbering of the respective sequences in the sequence listing. The wavy underlines in FIG. **3** indicate blanks which are considered in the IMGT numbering, although they are not occupied by amino acid residues. It is to be understood that Q44 refers to the IMGT numbering as shown in FIGS. **1** and **3**, not to the numbering as derivable from the attached sequence listing.

[0048] R7P1 D5, which has an α and β chain (TRAV5 and TRBV12-4), and TRAV 8-6 and TRBV 6-5, are only four examples of TCR variable domains that can be used in the context of the present invention. Other TRAV and TRBV subgroups are disclosed on the IGMT website. Note that the TRAV and TRBV can adopt specificity for different target epitope/MHC complexes by suitable adaptation of the CDR sequences, in particular.

[0049] Note also that the above mentioned sequences are shown without signal sequences, and that sometimes, the sequence databases show slightly deviating sequences. In the Uniprot database, for example, TRAV8-6 lacks the N-terminal A shown in SEQ ID NO: 3 as well as in FIG. **1**, while TRBV6-5 lacks the N-terminal N, and A shown in SEQ ID NO: 4, as well as in FIG. **1**.

[0050] According to another embodiment of the recombinant T cell receptor (TCR) heterodimer of the invention, the TCR includes one of the preferred substitution pairs selected from the following lists:

[0051] αQ44D/βQ44R; αQ44R/βQ44D; αQ44E/βQ44K; αQ44K/βQ44E; αQ44D/βQ44K; αQ44K/βQ44D; αQ44E/βQ44R; αQ44R/βQ44E; αQ44L/βQ44W; αQ44W/βQ44L; αQ44V/βQ44W; and αQ44W/βQ44V;

[0052] αW44D/βQ44R; αW44R/βQ44D; αW44E/βQ44K; αW44K/βQ44E; αW44D/βQ44K; αW44K/βQ44D; αW44E/βQ44R; αW44R/βQ44E; αW44L/βQ44W; αW44/βQ44L; αW44V/βQ44W; and αW44/βQ44V;

[0053] αH44D/βQ44R; αH44R/βQ44D; αH44E/βQ44K; αH44K/βQ44E; αH44D/βQ44K; αH44K/βQ44D; αH44E/βQ44R; αH44R/βQ44E; αH44L/βQ44W; αH44W/βQ44L; αH44V/βQ44W; and αH44W/βQ44V;

[0054] αK44D/βQ44R; αK44R/βQ44D; αK44E/βQ44K; αK44/βQ44E; αK44D/βQ44K; αK44/βQ44D; αK44E/βQ44R; αK44R/βQ44E; αK44L/βQ44W; αK44W/βQ44L; αK44V/βQ44W; and αK44W/βQ44V;

[0055] αE44D/βQ44R; αE44R/βQ44D; αE44/βQ44K; αE44K/βQ44E; αE44D/βQ44K; αE44K/βQ44D; αE44/βQ44R; αE44R/βQ44E; αE44L/βQ44W; αE44W/βQ44L; αE44V/βQ44W; and αE44W/βQ44V;

[0056] αQ44D/βR44; αQ44R/βR44D; αQ44E/βR44K; αQ44K/βR44E; αQ44D/βR44K; αQ44K/βR44D; αQ44E/βR44; αQ44R/βR44E; αQ44L/βR44W; αQ44W/βR44L; αQ44V/βR44W; and αQ44W/pR44V;

[0057] αW44D/βR44; αW44R/βR44D; αW44E/βR44K; αW44K/βR44E; αW44D/βR44K; αW44K/βR44D; αW44E/βR44; αW44R/βR44E; αW44L/βR44W; αW44/βR44L; αW44V/βR44W; and αW44/βR44V;

[0058] αH44D/βR44; αH44R/βR44D; αH44E/βR44K; αH44K/βR44E; αH44D/βR44K; αH44K/βR44D; αH44E/βR44; αH44R/βR44E; αH44L/βR44W; αH44W/βR44L; αH44V/βR44W; and αH44W/pR44V;

[0059] αK44D/βR44; αK44R/βR44D; αK44E/βR44K; αK44/βR44E; αK44D/βR44K; αK44/βR44D;

[0060] αK44E/βR44; αK44R/βR44E; αK44L/βR44W; αK44W/βR44L; αK44V/βR44W; and αK44W/pR44V;

[0061] αE44D/βR44; αE44R/βR44D; αE44/βR44K; αE44K/βR44E; αE44D/βR44K; αE44K/βR44D;

[0062] αE44R/βR44E; αE44L/βR44W; αE44W/βR44L; αE44V/βR44W; and αE44W/βR44V.

[0063] In the above, e.g. "αQ44R/βQ44D" shall mean for example that, in the variable domain of the α chain, Q44 is substituted by R, while in the variable domain of the β chain, Q44 is substituted by D.

[0064] According to preferred embodiments of said recombinant T cell receptor (TCR) heterodimer,

[0065] a) the modified α chain pairs preferably with the modified β chain, as compared to a non-modified β chain having Q or any other suitable amino acid at position 44 in the variable domain, and/or b) the modified β chain pairs preferably with the modified α chain, as compared to a non-modified α chain having Q or any other suitable amino acid at position 44 in the variable domain.

[0066] According to another aspect of the invention, a nucleic acid molecule encoding for a modified T cell receptor (TCR) α or β chain according to the above description and/or a recombinant T cell receptor (TCR) heterodimer according to the above description is provided. In one embodiment, the nucleic acid molecule further comprises at least one of a promoter operably linked to said one or more encoding nucleic acid molecules, and/or a signal sequence operably linked to said one or more encoding nucleic acid molecules.

[0067] The signal sequence encodes for a signal peptide that directs the α or β chain to the cellular surface of the T cells, where it is anchored by means of its transmembrane domain, the α and β chain being displayed on the cells outer surface. Signal sequences for the different α and β chain variable domain subtypes are disclosed in the art.

[0068] According to another aspect of the invention, a plasmid or vector comprising at least one of the nucleic acid molecules set forth above is provided.

[0069] In one embodiment, said vector is preferably a viral vector, preferably a retroviral vector or lentiviral vector. Methods of transducing α or β T cell receptor (TCR) genes into T cells with viral vectors are for example disclosed in Pogulis and Pease (1998), or Zong et al. (2010). The use of Lentiviral vectors for gene transfer into human T cells is disclosed in Verhoeyen et al. (2009).

[0070] In one other embodiment, said vector comprises a transposon, like piggyback or sleeping beauty, which in turn is capable of transferring the respective nucleic acid into the T cell. Methods of using transposons for genetically engineering T cells are for example disclosed in Huang et al. (2008).

[0071] In another embodiment, the T cell can be transiently transfected, e.g., by means of introducing one or more RNAs encoding for the α and β chains, e.g., by means of electroporation. Such methods are e.g. disclosed in Kim and Eberwine (2010).

[0072] According to another aspect of the invention, a method of preparing a modified T cell is provided, said method comprising the steps of:

[0073] obtaining a T cell from a subject, and transducing or transfecting said T cell with one or more nucleic acid molecules according to the present invention, or a plasmid or vector according to the present invention.

[0074] In a preferred embodiment, said T cell has been obtained from an HLA allele negative donor. For example, if the modified T cell is meant to detect antigens presented by a HLA-A*02 serotype APC, the source that is meant to be modified is preferably obtained from a HLA-A*02 serotype negative donor. In such manner, cross reactivity of the endogenous TCR with the target antigen/MHC complex is reduced, or even avoided.

[0075] Preferably, the modified T cell obtained in such way is suitable for autologous T cell treatment of said subject.

[0076] According to another aspect of the invention, a modified T cell bearing a set of nucleic acids encoding for the α and β chains of a recombinant T cell receptor (TCR) heterodimer according to the above description is provided.

[0077] In one embodiment, said cell has been prepared by a method according to the above description.

[0078] According to another aspect of the invention, the use of a modified T cell according to the above description for the treatment of a patient that is suffering from, at risk of developing, and/or being diagnosed for a neoplastic, inflammatory, infectious or autoimmune disease is provided, which use comprises providing or administering to a patient in need thereof a preparation comprising said modified T cell.

[0079] Alternatively, a method of treating a patient that is suffering from, at risk of developing, and/or being diagnosed for a neoplastic, inflammatory, infectious or autoimmune disease, with a modified T cell according to the above description is provided, which method comprises providing or administering to a patient in need thereof a preparation comprising said modified T cell.

[0080] It is, in this context, important to understand that the specificity of the respective modified TCR, or of the respective modified T cell, is dependent on CDR sequences in the variable domains of the α and β chains, in order to detect antigens presented by the MHC of an antigen presenting cells.

[0081] As described in Løset et al. (2014), T cell receptors for a specific antigen-MHC complex can be obtained by T cell related phage display. In such approach, the Q44 substitutions in the variable domains of α and β chain can either be accomplished in all members of the library that forms the basis for the phage display, or can be introduced afterwards, i.e., once a specific TCR that detects a specific antigen-MHC complex has been found.

[0082] According to further aspects of the invention, the use, method, T cell or T cell receptor (TCR) heterodimer according to the above description is provided, wherein the antigen presented by the MHC complex detected by the T cell receptor is selected from a cancer specific tumor associated antigen (TAA) peptide epitopes. These peptides are known in the art, and comprise, as an example, peptide MAG-003 as used in the examples. These peptides can be found in the literature, for example the Epitope Database (www.iedb.org/) or preferred as disclosed in any of WO 2016/177784; WO 2016/170139; WO 2016/156230; WO 2016/156202; WO 2016/146751; WO 2016/102272; WO 2016/107740; WO 2015/193359; WO 2015/169945; WO 2015/063302; WO 2015/018805; WO 2012/069462; WO 2012/079878; WO 2012/038463; WO 2011/151403; WO 2011/128448; WO 2011/113882; WO 2011/113872; WO 2011/113819; WO 2011/073215; WO 2010/037514; WO 2009/138236; WO 2007/028574; WO 2007/028573; WO 2006/114307; WO 2005/116051; WO 2005/076009; WO 2004/085461; WO 03/100432; WO 03/102023; WO 2009/015843; WO 2009/015842; WO 2009/015841; WO 2016/202963; WO 2016/207164; WO 2017/001491; WO 2017/005733; WO 2017/021527; WO 2017/036936; WO 2017/060169; WO 2017/060201; WO 2017/097602; WO 2017/

097699; WO 2017/108345; or WO 2017/009400 (all hereby incorporated by reference with regards to the disclosed peptides).

[0083] While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive; the invention is not limited to the disclosed embodiments. Other variations to the disclosed embodiments can be understood and effected by those skilled in the art in practicing the claimed invention, and/or from studying the drawings, the disclosure, and the appended claims.

[0084] It is further to be understood that this invention is not limited to the particular component parts or structural features of the devices or compositions described or process steps of the methods described as such devices and methods may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. Any reference signs in the claims should not be construed as limiting the scope. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include singular and/or plural referents unless the context clearly dictates otherwise. Further, in the claims, the word "comprising" does not exclude other elements or steps. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. It is moreover to be understood that, in case parameter ranges are given which are delimited by numeric values, the ranges are deemed to include these limitation values.

[0085] It is further to be understood that embodiments disclosed herein are not meant to be understood as individual embodiments which would not relate to one another. Features discussed with one embodiment are meant to be disclosed also in connection with other embodiments shown herein. If, in one case, a specific feature is not disclosed with one embodiment, but with another, the skilled person would understand that does not necessarily mean that said feature is not meant to be disclosed with said other embodiment. The skilled person would understand that it is the gist of this application to disclose said feature also for the other embodiment, but that just for purposes of clarity and to keep the specification in a manageable volume this has not been done.

[0086] All amino acid sequences disclosed herein are shown from N-terminus to C-terminus; all nucleic acid sequences disclosed herein are shown 5'->3'. For the purposes of the present invention, all references as cited herein are incorporated by reference in their entireties. This refers, particularly, for prior art documents that disclose standard or routine methods. In that case, the incorporation by reference has mainly the purpose to provide sufficient enabling disclosure, and avoid lengthy repetitions.

[0087] In the figures and the attached sequence listing, FIGS. **1**A to C show the numbering of amino acid residues in the variable domains of TCR α chain and β chain. Figure taken and modified from Lefranc et al. (2003). The sequences are shown on the example of TRAV8-6 (Gene ID: 28680) (alpha chain) (SEQ ID NO: 3) and TRBV6-5 (Gene ID: 28602) (beta chain) (SEQ ID NO: 4). The numbering of the amino acid residues in the α chain and β chain is according to the IMGT standard (bold), compared to the Kabat numbering. In FIG. **1** B, Q44 in the α chain (TRAV) and β chain (TRBV) are marked with a grey underlay. Q44 according to the IMGT numbering is a highly conserved residue in the Framework 2 (FR2) region and shared by about 80% of all TCR genes (Strong et al., 1999), including 1G4 (pdb ID: 2BNR (Chen et al., 2005)); TRAV8-6 (Gene ID: 28680) (α chain) (SEQ ID NO: 3) and TRBV6-5 (Gene ID: 28602) (SEQ ID NO: 4), (β chain) and R7P1 D5 (comprising TRAV5 (SEQ ID NO: 1) and TRBV12-4 (SEQ ID NO: 2), Lefranc et al. 2001). Q44 is very often part of the 4 AA motif comprising WYXQ, with X being any amino acid, for example, R, V or K. W41 is even more conserved (>95%) in TCR α and β chains (Strong et al., 1999).

[0088] FIG. **2**A shows the structural motif of the αQ44/βQ44 from the variable domain of the TCR 1 G4 (pdb ID: 2BNR (Chen et al., 2005)). FIG. **2**B shows in silico double mutants, based on the crystal structure of the 1G4 TCR. From the wild type αQ44/βQ44 pair, the inventors manually selected and engineered potential pairs that maintain a high level of molecular contacts (polar or apolar), while breaking the sterical and/or charge symmetry. The mutants were created with UCSF Chimera (Pettersen et al., 2004). Throughout the whole figures, the TCR α and β chains are represented in dark blue and cyan ribbons, respectively. The side chains of interest are highlighted in magenta and all heavy atoms are shown.

[0089] FIG. **3** shows sequences of TCR variants R7P1 D5 α and β chains (TRAV5 and TRBV12-4), detects a peptide called MAG-003 when bound to the MHC; TRAV8-6 (α chain variable domain); and TRBV6-5 (13 chain variable domain). The grey underlay marks the approximate positions of CDR1 (SEQ ID NO: 5 for TRAV5, SEQ ID NO: 8 for TRBV12-4, SEQ ID NO: 11 for TRAV8-6, and SEQ ID NO: 14 for TRBV6-5), CDR2 (SEQ ID NO: 6 for TRAV5, SEQ ID NO: 9 for TRBV12-4, SEQ ID NO: 12 for TRAV8-6, and SEQ ID NO: 15 for TRBV6-5) and CDR3 (SEQ ID NO: 7 for TRAV5, SEQ ID NO: 10 for TRBV12-4, SEQ ID NO: 13 for TRAV8-6, and SEQ ID NO: 16 for TRBV6-5), with the framework regions FR1, FR2 and FR3. As can be seen, Q44 is located in FR2. Note that Q44 is conserved in other TCR variants, too, just like in TRAV8-6 and TRBV6-5. As in the latter, Q44 is in R7P1 D5 part of a 4 AA motif comprising WYXQ. Depending on the respective antigen that is targeted, the CDR sequences can of course vary.

[0090] FIG. **4**A and FIG. **4**B show the computed mutation energies for selected in silico engineered mutants. Double mutations are selected for a resulting shape and/or charge complementarity at the TRAV/TRBV interface. FIG. **4**A shows a selection of charged amino acids (D, E, K, R) while FIG. **4**B shows a selection of neutral amino acids (W, V, L). For each suggested pair of complementary mutations, the inventors tested the double mutant (green), as well as each individual single mutant (orange) to mimic the pairing of an engineered chain with a wild type chain. Mutation energies higher than 0.5 kcal/mol are considered destabilizing, mutation energies lower than −0.5 kcal/mol are considered stabilizing, mutation energies between −0.5 and 0.5 kcal/mol are considered neutral (in between red lines—software default parameters). The mutations were performed on the variable domain of the 1 G4 TCR (Chen et al., 2005) with

Discovery Studio (Dassault Systemes, BIOVIA, 2017) and the mutation energy algorithm described by Spassov and Yan (2013).

[0091] FIG. **5** shows MAG-003 (exemplary peptide): HLA-A*02 tetramer or NYESO1-001 (control peptide): HLA-A*02 tetramer staining, respectively, of CD8+ T-cells electroporated with alpha and beta chain RNA of TCR R7P1 D5 wt and mutant variants. R7P1 D5 detects a MAG-003 peptide when bound to the MHC, but does not detect a NYESO1 peptide. Mock-electroporated CD8+ T-cells (no TCR) served as controls. Donors (left panel: donor A, right panel: donor B).

[0092] FIG. **6** shows IFN release with MAG-003 as an exemplary peptide in the HLA-A*02 context of CD8+ T-cells that were electroporated with alpha and beta chain RNA of TCR R7P1 D5 wt and mutant variants. All mutant variants (αQ44R/βQ44D, αQ44E/βQ44K, αQ44K/βQ44E, αQ44D/βQ44K, αQ44K/βQ44D, αQ44E/βQ44R) show an improved MAG-003 recognition when compared to unmodified R7P1 D5 wt.

[0093] In FIG. **7**, PRAME-004 specific TCRs R11A and R17A and their corresponding a44K/344E mutant variants, respectively were transduced into human T cell via lentiviral transfer. The activity of TCR-transduced T cells was assessed by co-incubation with T2 cells loaded with decreasing concentration of PRAME-004 peptide. Mutant TCR R11 KEA shows vastly improved PRAME-004 recognition when compared to unmodified R11 TCR.

[0094] In FIG. **8**, PRAME-004 specific TCR R17A and R11A and its corresponding 44K/44E mutant variant (R11 KEA) were transduced into human T cell of two donors via lentivirus transfer. Cytotoxicity of transduced T cells against of PRAME-004 expressing tumor cell lines A375 and U20S was assessed via IncuCyte imaging system. MAG-003 specific TCR was used as control (A375 and U20S express MAG-003 as well). Mutant TCR R11 KEA show increased killing of PRAME-004 positive cell lines when compared to unmodified R11ATCR.

EXAMPLES

[0095] Note that the numbering according to the IMGT standard may deviate from the naïve numbering of a given amino acid sequence of a TCR variable domain, in particular due to blanks in the CDR sequences, as can be seen in FIG. **1** A-C. This applies, e.g., for TRAV8-6 and TRBV6-5, where the wavy underlines show blanks which are considered in the IMGT numbering, although they are not occupied by amino acid residues. These sequences are provided as examples only, and—although preferred—shall not restrict the claims to specific embodiments. This means that the teaching of the present invention is applicable to other TCR α and β chains, or TCR αβ heterodimers, having other sequences, in particular in the variable domain, in particular in the CDRs.

[0096] Further, the teaching of the present invention is also applicable to other TCR α and β chains or TCR αβ heterodimers binding to other target antigen/MHC complexes as well, preferably, but not only, when comprising the natural Q44 amino acid, and preferably when they comprise a WYXQ motif. Depending on the respective antigen as targeted, the CDR sequences may vary.

[0097] In general, methods of T-Cell Receptor Cloning and Expression have been disclosed in Walchli et al. (2011). Methods of random mutagenesis or site directed mutagenesis leading to site-specific amino acid substitutions are well known to the skilled person, and are for example disclosed in Labrou (2010) and Trehan et al. (2016).

[0098] Methods of genome editing to modify a given amino acid sequence are well known to the skilled person (e.g., CRISPR Cas, TALEN, ZFN, Argonaute (NgAgo) or CRISPR Cpf1), and are for example disclosed in Maeder and Gersbach (2016). Methods of synthesizing genes are well known to the skilled person, and are for example disclosed in Hughes et al. (2011). The disclosure of these references is incorporated by reference in their entireties.

In Silico Methods

[0099] By visually inspecting the variable domain of the 1 G4 TCR (pdb ID: 2BNR (Chen et al., 2005)), the inventors manually selected pairs of mutations for the α44/β44 motif, that would potentially maintain a high level of molecular contacts (polar or apolar), while breaking the steric and/or charge symmetry. Mutation pairs were selected such that (i) the total charge of the pair was zero, (ii) the two amino acids would potentially show a good shape complementarity and/or form hydrogen bond and/or salt bridges, and (iii) the two amino acids would have a different molecular weight (i.e. one large amino acid and one small amino acid). The Discovery Studio software (Dassault Systemes, BIOVIA, 2017) was used to investigate further the effect of the engineered positions on the αβ pairing of the TCR. Mutation energies were computed by using the algorithm described by Spassov et al. (Spassov and Yan, 2013) to perform in silico design of antibodies. Mutation energies were expected reflect the effect of a mutation, compared to the wild type motif αQ44/βQ44. The inventors tested double mutants as well as each single mutant paired to a wild type chain:

[0100] in case of double mutants, the mutation energy was expected to be neutralor stabilizing

[0101] in case of single mutants paired to a wild type chain, the mutation energy was expected to be destabilizing for at least one of the two sides.

[0102] Human primary CD8+ T cells were electroporated without RNA (no TCR) or with the same amount of RNA encoding for peptide-specific (MAG-003 as an example, peptide "p286" as disclosed, e.g., by Wu et al. Scandinavian journal of immunology 74:6 2011 December pages 561-7) T cell receptor chains α and β in its wild type form (R7P1 D5 TCR wt) or in a mutant form (R7P1 D5 TCR αQ44D/βQ44R) incorporating a Q44D mutation in the TCR alpha variable domain and a Q44R mutation in the TCR beta variable domain. After overnight cultivation the T cells were analyzed for binding of MAG-003:HLA-A*02 tetramers (FIG. **5**, upper row) and binding of unrelated peptide NYESO1-001:HLA-A*02 control tetramers (FIG. **5**, lower row)(peptide NYESO-001; Epitope ID 59283 in the Epitope Database as above). The proportion of tetramer positive T cells is show for two individual donors (FIG. **5** left panel: donor A, right panel: donor B).

[0103] The TCR R7P1 D5, encoding a tumor specific TCR-alpha and TCR-beta chain, was isolated and amplified from T-cells of a healthy donor. Cells from healthy donors were in vitro stimulated according to a method previously described (Walter et al., 2003) and target-specific cells were single-cell sorted using HLA-A*02 multimers and then used for subsequent TCR isolation. TCR sequences were isolated via 5' RACE by standard methods as described by e.g. Molecular Cloning a laboratory manual fourth edition by

Green and Sambrook. The alpha and beta variable regions of TCR R7P1 D5 was sequenced and cloned for further functional characterization. TCR R7P1 D5 is derived from a HLA-A*02 positive donor.

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SEQUENCE LISTING

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mol_type = protein
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source 1..94
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organism = synthetic construct
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source 1..95
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INGFEAEFNK SQTSFHLRKP SVHISDTAEY FCAVS 95

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PNGYNVSRST TEDFPLRLLS AAPSQTSVYF CASSY 95

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source                  1..5
                        mol_type = protein
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source                  1..4
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VARIANT                 2
                        note = Val or Leu or Ala
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source                  1..9
                        mol_type = protein
                        organism = synthetic construct
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source                  1..282
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 18
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ctgaggtgca actactcatc gtctgtttca gtgtatctct tctggtatgt gcaatacccc  120
aaccaaggac tccagcttct cctgaagtat ttatcaggat ccaccctggt tgaaagcatc  180
aacggttttg aggctgaatt taacaagagt caaacttcct tccacttgag gaaaccctca  240
gtccatataa gcgacacggc tgagtacttc tgtgctgtga gt                     282

SEQ ID NO: 19           moltype = DNA  length = 285
FEATURE                 Location/Qualifiers
misc_feature            1..285
                        note = TCR beta chain variable domain of TRBV 6-5
source                  1..285
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 19
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atggggctga ggctgattca ttactcagtt ggtgctggta tcactgacca aggagaagtc  180
cccaatggct acaatgtctc cagatcaacc acagaggatt tcccgctcag gctgctgtcg  240
gctgctccct cccagacatc tgtgtacttc tgtgccagca gttat                  285
```

1. A modified T cell receptor (TCR) α or β chain, or a fragment or derivative thereof that maintains the ability to bind to an antigen-MHC complex, wherein the amino acid at position 44 according to the IMGT numbering of a non-modified chain is substituted with another suitable amino acid that reduces the pairing of said modified α or β chain with an undesired α or β chain.

2. The modified T cell receptor (TCR) α or β chain of claim **1**, wherein said undesired α or β chain is non-modified.

3. The modified T cell receptor (TCR) α or β chain of claim **1**, wherein said amino acid at position 44 in the variable domain is substituted by an amino acid selected from the group consisting of the amino acids Q, R, D, E, K, L, W, and V.

4. The modified T cell receptor (TCR) α or β chain according to claim **1**, wherein the modified α chain has a variable domain sequence comprising at least the framework regions of a sequence having at least 95% sequence identity with SEQ ID NO: 1 or 3, and/or wherein the modified β chain has a variable domain sequence comprising the framework regions of a sequence having least 95% sequence identity with SEQ ID NO 2 or 4.

5. A recombinant T cell receptor (TCR) heterodimer comprising a modified α chain and/or a modified β chain, or fragments or derivatives thereof according to any claim **1**, wherein said TCR maintains the ability to bind to an antigen-MHC complex.

6. The recombinant T cell receptor (TCR) heterodimer according to claim **5**, wherein said modification of the α and/or β chain thereof is selected from a substitution of position 44 by an amino acid selected from the group consisting of Q, R, D, E, K, L, W, and V.

7. The recombinant T cell receptor (TCR) heterodimer according to claim **5**, wherein said modification of the α and β chain is selected from a substitution pair of the group consisting of αQ44D/βQ44R; αQ44E/βQ44K; αQ44E/βQ44R; αQ44R/βQ44E; αQ44L/βQ44W; αQ44W/βQ44L; αQ44V/βQ44W; αQ44W/βQ44V; αW44D/βQ44R; αW44R/βQ44D; αW44E/βQ44K; αW44D/βQ44K; αW44K/βQ44D; αW44E/βQ44R; αW44R/βQ44E; αW44L/βQ44W; αW44/βQ44L; αW44V/βQ44W; αW44/βQ44V; αH44D/βQ44R; αH44R/βQ44D; αH44E/βQ44K; αH44K/βQ44E; αH44D/βQ44K; αH44K/βQ44D; αH44E/βQ44R; αH44R/βQ44E; αH44L/βQ44W; αH44W/βQ44L; αH44V/βQ44W; αH44W/βQ44V; αK44D/βQ44R; αK44R/βQ44D; αK44E/βQ44K; αK44/βQ44E; αK44D/βQ44K; αK44/βQ44D; αK44E/βQ44R; αK44R/βQ44E; αK44L/βQ44W; αK44W/βQ44L; αK44V/βQ44W; αK44W/βQ44V; αE44D/βQ44R; αE44R/βQ44D; αE44/βQ44K; αE44D/βQ44K; αE44K/βQ44D; αE44/βQ44R; αE44R/βQ44E; αE44L/βQ44W; αE44W/βQ44L; αE44V/βQ44W; αE44W/βQ44V; αQ44D/βR44; αQ44R/βR44D; αQ44E/βR44K; αQ44D/βR44K; αQ44K/βR44D; αQ44E/βR44; αQ44R/βR44E; αQ44L/βR44W; αQ44W/βR44L; αQ44V/βR44W; αQ44W/βR44V; αW44D/βR44; αW44R/βR44D; αW44E/βR44K; αW44D/βR44K; αW44K/

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8. The recombinant T cell receptor (TCR) heterodimer according to claim **5**, wherein said α chain of the TCR has a variable domain sequence comprising at least the framework regions of a sequence having at least 95% sequence identity with SEQ ID NO: 1 or 3, and/or the β chain of the TCR has a variable domain sequence comprising the framework regions of a sequence having at least 95% sequence identity with SEQ ID NO: 2 or 4.

9. A nucleic acid molecule encoding for a modified T cell receptor (TCR) α or β chain according to claim **1**, and/or a recombinant T cell receptor (TCR) heterodimer comprising said modified α chain and/or a modified β chain, or fragments or derivatives thereof, wherein said TCR maintains the ability to bind to an antigen-MHC complex.

10. A plasmid or expression vector comprising at least one of the nucleic acid molecules according to claim **9**.

11. A method of preparing a modified T cell, said method comprising transducing or transfecting a T cell obtained from a subject with one or more nucleic acid molecules according to claim **9**, or a plasmid or expression vector comprising at least one of the nucleic acid molecules.

12. A modified T cell produced according to claim **11**.

13. The modified T cell according to claim **12** for use in an autologous T cell treatment of a subject in need thereof.

14. The modified T cell according to claim **12** for use in the treatment of a subject in need of said treatment that is suffering from or is at risk of developing, and/or is diagnosed with a neoplastic, inflammatory, infectious or autoimmune disease, comprising administering to said subject in need thereof a preparation comprising said modified T cell.

15. The recombinant T cell receptor (TCR) heterodimer according to claim **5**, wherein said TCR specifically binds to an antigen presented by an MHC complex, wherein said antigen is selected from an epitope of a tumor associated antigen (TAA).

16. A recombinant TCR comprising an α chain or a fragment thereof comprising a variable domain and a β chain or a fragment thereof comprising a variable domain that maintains the ability to bind to an antigen-MHC complex, wherein the α chain and the 1 chain comprises α44D/β44R; α44R/β44D; α44E/β44K; α44D/β44K; α44K/β44D; α44E/β44R; or α44R/β44E.

17. A nucleic acid molecule encoding the recombinant TCR of claim **16**.

18. A plasmid or an expression vector comprising the nucleic acid of claim **17**.

19. A method of preparing a T cell, comprising transducing or transfecting a T cell obtained from a subject with the plasmid or the expression vector of claim **18**.

20. A T cell produced by the method of claim **19**.

21. A composition comprising the modified TCR of claim **1**.

22. A composition comprising the recombinant TCR of claim **16**.

* * * * *